

Occupational asthma and immunologic responses induced by inhaled carmine among employees at a factory making natural dyes

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Abstract

Carmine is a natural red dye widely used as a food coloring agent and for cosmetic manufacture. It is extracted from the dried females of the insect *Dactylopius coccus* var. Costa (cochineal). Although it has been reported that inhalation of carmine may give rise to occupational asthma and extrinsic allergic alveolitis, there is little evidence of its immunogenic capacity. We studied nine current employees at a factory making natural dyes and one former employee who had left this plant after occupational asthma developed. A current employee had work-related symptoms of rhinitis and asthma that were confirmed by bronchial provocation tests, and another worker had rhinitis. Immunologic sensitization to carmine and cochineal was evaluated by means of skin testing and determination of serum-specific IgE and IgG subclass antibodies by RAST and ELISA, respectively. The specificity of the RAST assay was investigated by RAST inhibition with different fractions of carmine. The three workers with respiratory symptoms had positive skin prick test reactions to both carmine and cochineal. An immediate response to the bronchial provocation test with carmine and cochineal was observed in the current employee with asthma. Specific IgE antibodies against carmine and cochineal were found only in this worker. RAST inhibition studies indicated that the main allergen had a molecular weight between 10 and 30 kd. Specific IgG antibodies against carmine and cochineal, mainly the subclasses IgG₁, IgG₃, and IgG₄, were found in the 10 subjects surveyed. These findings suggest that carmine may induce immunologic responses, most

likely IgE mediated in workers with symptoms of occupational asthma. (J ALLERGY CLIN IMMUNOL 1994;93:44-52.)

BPT	Bronchial provocation test
CI	Color Index number
FEV₁	Forced expiratory volume in 1 second
HSA	Human serum albumin
MW	Molecular weight
PBS	Phosphate-buffered saline solution
PD₂₀	Provocative dose of methacholine causing a 20% fall in FEV ₁
PEFR	Peak expiratory flow rate

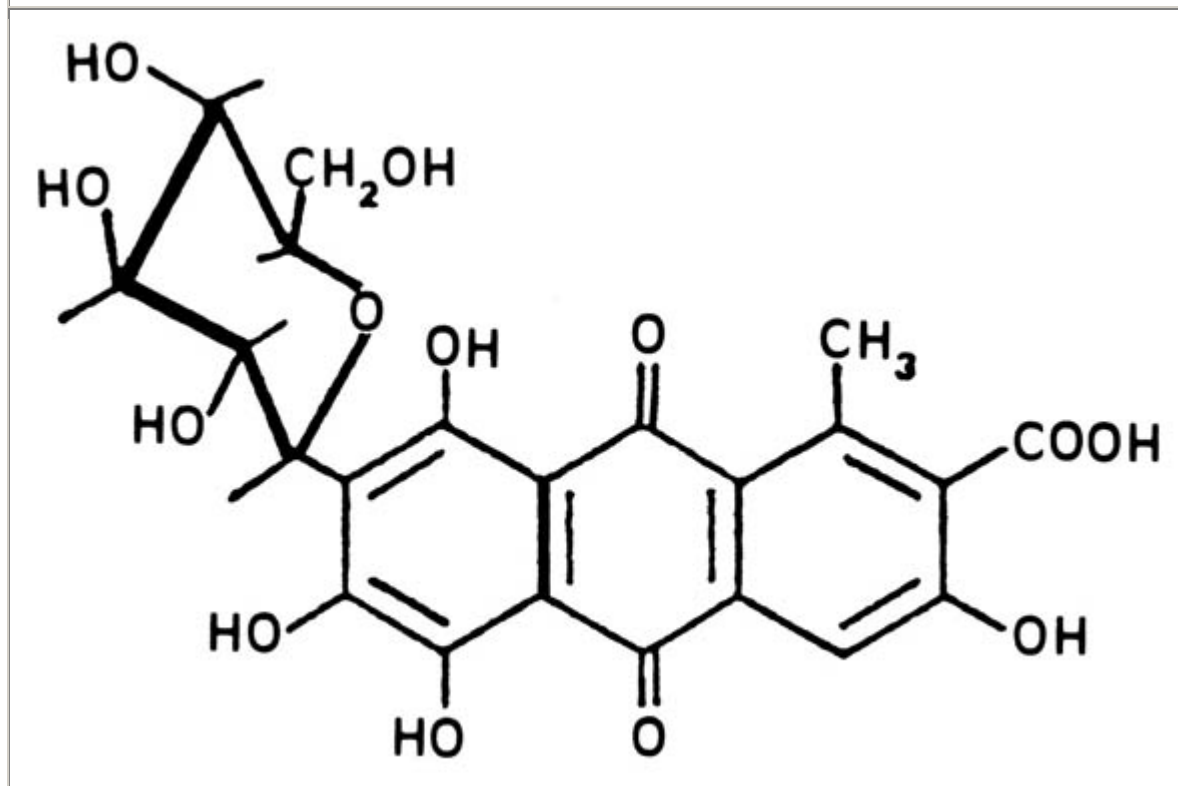
Key words: [Annatto](#), [anthraquinones](#), [arthropods](#), [bronchial provocation test](#), [carmine](#), [cochineal](#), [curcuma](#), [dyes](#), [occupational asthma](#), [RAST](#)

It is known that exposure to reactive dyes may give rise to rhinitis and occupational asthma. ¹⁻⁴ IgE antibodies specific to these reactive low-molecular weight (MW) compounds have been demonstrated by both positive skin prick test results and optimized RAST. ⁵ Although it has also been reported that the natural dye carmine can cause either occupational asthma ^{6,7} or extrinsic allergic alveolitis, ^{8,9} little is known about the underlying pathogenic mechanisms and the immunologic responses to this dye in exposed workers.

Carmine (Color Index No. [CI] 75470) is a natural red colorant obtained from aqueous extracts of cochineal, which consist of the dried bodies of the female insect *Dactylopius coccus* var. Costa (family Dactylopiidae, superfamily Coccoidea). This insect, which lives as a parasite on some species of cacti, particularly *Nopalea cochenillifera*, originated from Central and South America. In Europe cochineal raising is restricted to the Canary Islands of Spain. The coloring principle is a hydrated aluminum chelate of carminic acid (MW 492.4 d) (■Fig. 1). The process of obtaining carmine comprises aqueous extraction of cochineal, precipitation of carmine with sodium sulfate and calcium carbonate, filtration, sterilization, and drying; it is then milled, mixed (usually with sucrose to adjust color by spectrophotometry on the basis of carminic acid content), and finally bagged. We observed in the factory surveyed that a highly dusty atmosphere of carmine powder is generated during these processes, mainly during the milling, mixing, and packing stages; for this reason the process workers wear face masks. Carmine is among the most interesting color additives in use today not only because of its characteristics but also because of the part its source, cochineal, played in the political and economic history of the New World and on those who settled there. Today carmine is used in the food (mainly

in butchery and confectionery), cosmetic, and pharmaceutical industries, and also as an histologic dye.

FIG. 1. Structural formula of carminic acid (class anthraquinone).



We report the clinical and immunologic studies carried out on nine current employees and one former worker at a natural dye factory. The main dye elaborated in this factory was carmine, although other natural colorants such as annatto and curcuma (turmeric, or Indian saffron) were also produced. Annatto (CI 75120) is an orange-yellow food coloring extracted from the pericarp of the seeds of the tree *Bixa orellana*; the carotenoid norbixin (MW 380.48 d) is the primary pigment. Curcuma (CI 75300) is a yellow-brown food coloring with a characteristic flavor and taste and is obtained from the ground rhizome of *Curcuma longa*. Its coloring principle is curcumin (MW 368.4 d).

METHODS

Subjects

A study was carried out on the nine current employees and one former worker (previously affected by carmine-induced asthma) at a factory involved in the production of dyes from natural sources, mainly cochineal carmine but also annatto and curcuma. Although these dyes were produced in batches, carmine was elaborated continuously because of the high current trade demand. In a clinical questionnaire the workers noted the existence of any respiratory (nasal or bronchial) symptoms, the possible relationship with the working environment, the symptoms observed in vacation periods or during absence from work, the existence of allergic history, occupation, and level of exposure.

All workers underwent skin tests and baseline pulmonary function tests; and a blood sample was obtained for specific antibody measurements.

Dye extracts for in vivo and in vitro tests

Cochineal (dried female insects) purchased from the Canary Islands and Peru, water-soluble carmine containing 52% carminic acid, and annatto and curcuma (in powder form) were all obtained from the factory surveyed. Carminic acid was supplied by Sigma Chemical Co., St. Louis, Mo.

Untreated cochineal was ground to a powder, and then a 1:10 wt/vol extract was prepared by dissolving 2 gm of this material in 20 ml of phosphate-buffered saline solution (PBS) (pH 7.3) at room temperature. The resulting suspension was stirred for 1 hour and then centrifuged (3000 g for 15 minutes). The supernatant was passed through filter paper and then through a 0.22 μ m Millipore filter (Millipore Corp., Bedford, Mass.) for sterilization. Annatto and curcuma extracts, 1:10 wt/vol, were obtained by the same protocol. Extracts of carmine and carminic acid were prepared at a concentration of 10 mg/ml in PBS. Carminic acid-human serum albumin (HSA) conjugate was obtained according to the method of Levine and Ovary.¹⁰ Protein concentration of carmine and cochineal extracts was determined by Bio-Rad protein assay (Bio-Rad, Munich, Germany); this concentration was 2.8 mg/ml in carmine extract and 1.03 mg/ml in cochineal extract. Several 10-fold dilutions of these extracts were done for skin and inhalation tests.

Skin prick tests

Skin prick tests were performed with the extracts described previously. A battery of common inhalant allergens, including *Dermatophagoides pteronyssinus*, cockroach, grass and plantain pollen, *Aspergillus spp.*, *Alternaria alternata*, *Cladosporium herbarum*, *Mucor racemosus*, *Penicillium notatum*, and dog and cat epithelia (Abelló S.A., Madrid, Spain), was also tested. Histamine phosphate, 10 mg/ml, and PBS were used as positive and negative controls. Responses were examined after 15 minutes by measuring the two perpendicular diameters of the wheal. A positive response was defined as a wheal with a mean diameter greater than 3 mm in the presence of a negative response to the diluent.

Fifteen atopic nonexposed persons and 15 nonallergic patients from our clinic, who served as a control group, were tested with these dye extracts to determine nonirritating concentrations.

Total IgE

Total IgE levels in the subjects' sera were measured with the Pharmacia CAP system IgE fluoro-enzyme-immunosorbent-assay (Pharmacia Diagnostics, Uppsala, Sweden).

Specific IgE determination by RAST

To detect specific IgE antibodies against cochineal and carmine, we treated cyanogen bromide-activated cellulose disks (Pharmacia Diagnostics) with our own extracts. The extracts of cochineal (1:10 wt/vol), carmine (10 mg/ml), and carminic acid-HSA conjugate were added to the disks, and after an overnight incubation period at 24° C, the disks were washed three times in PBS 0.1% containing 0.1% Tween 20 (polysorbate 20) (PBS-TW). Bovine serum albumin, 1% in PBS, was then added and the disks were incubated for 1

hour at 24° C. After the disks were washed three more times with PBS-TW, they were ready for use. They were stored in PBS at 4° C until needed. RAST was conducted according to the standard technique, which uses iodine 125-labeled rabbit antihuman IgE (Pharmacia Diagnostics). Briefly, 50 µl of each undiluted serum was added to each disk, incubated for 3 hours at 24° C, and washed three times with Pharmacia RAST washing solution; 50 µl of ¹²⁵I-radiolabeled anti-human IgE with an activity of approximately 40,000 counts/min was then added. After overnight incubation at 24° C, the disks were washed three times and counted in a gamma counter. The sera were added in duplicate, and the results were expressed as the mean of the percentage of the total activity added. The lower limit for a positive test result was defined according to the Phadebas RAST reference system (Pharmacia Diagnostics). This limit (RAST class 1) was 3.5% of the total activity added. Sera from 10 atopic patients were used as controls.

RAST inhibition assays

Competitive RAST inhibition was used to investigate the specificity of the carmine RAST and the existence of shared antigens between carmine and cochineal. Twenty-five microliters of three progressive 10-fold dilutions of the inhibitor allergens in PBS (the most concentrated having a protein concentration of 1 mg/ml) and 25 µl of PBS as negative control were incubated with 50 µl of serum from subject 1 for 1 hour at 24° C as liquid phase. Then our own disks coupled to carmine were added as solid phase. After incubation for 3 hours, the assay was continued with the standard RAST technique. The following inhibitors were used: carmine, cochineal, and carmine fractions of different MW (>30 kd and < 30 kd, and > 10 kd and < 10 kd). These carmine fractions were obtained by passing the carmine extract (10 mg/ml) through the Centricon (Amicon, Danvers, Mass.) Centricon-30 (30 kd MW cutoff), and Centricon-10 (10 kd MW cutoff) filters, respectively. Results were expressed as the percent inhibition of radiolabeled anti-IgE bound as compared with a control reaction with PBS inhibitor.

Specific IgG subclasses by indirect ELISA

Indirect ELISA detects the presence of specific IgG subclass antibodies with the use of allergen-coated Costar 3590 microtiter plates (Costar Corp., Cambridge, Mass.). Briefly, 0.1 of either carmine, 0.1 mg/ml, or cochineal, 1:1000 wt/vol in PBS, was added to coat each microtiter plate well. After the wells were coated, 50 µl of each serum diluted 1:2 in PBS-TW was incubated in the wells for 18 hours at 4° C. After plates were washed with PBS-TW, 1:4000 peroxidase-labeled anti-human IgG₁ and 1:1000 peroxidase-labeled anti-human IgG₂, IgG₃, and IgG₄ (Tago Laboratories, Burlingame, Calif.) diluted in 10% fetal calf serum in PBS-TW diluted in PBS-T containing 10% fetal calf serum were added to the wells and incubated for 3 hours at 24° C. The wells were then washed in PBS-TW, and 0.2 ml of substrate was added (o-phenylenediamine, 2 mg/ml, in enzyme buffer with 0.1 mol/L citrate, pH 5, containing 0.03% hydrogen peroxide) at 24° C. After 15 minutes the color reaction was arrested by the addition of 4N sulfuric acid. Optical density at 492 nm was measured with a Titertek-Multiscan (Flow Laboratories, Irvine, Scotland). The indirect ELISA was performed in duplicate for each specific immunoglobulin. The control group consisted of sera from seven nonexposed persons. A positive result was considered as a value higher than the mean of the control sera + 2 standard deviations. The specificity of the ELISA assays for carmine and cochineal IgG subclasses was evaluated by ELISA inhibition by preincubating 50 µl (1:2 in PBS-TW) of a pooled serum from the study subjects with 25 µl of the corresponding undiluted allergen extract for 1 hour at 24° C, and then the procedure was followed as mentioned previously.

Ambulatory monitoring of peak expiratory flow rate

Basal peak expiratory flow rate (PEFR) measurements from all workers were obtained with a Mini-Wright peak flow meter (Clement Clarke International Ltd., London, UK). Serial determinations of PEFR were made only by the worker with asthma (subject 1) at 2-hour intervals during his waking hours at work and at home for 4 weeks. The maximum and minimum values were kept for analysis of daily variability. Records with a diurnal variation equal to or greater than 20% were considered significant for daily variability.

Bronchial provocation tests

Methacholine inhalation was performed in subject 1 according to the method described by Chai et al. ¹¹ Methacholine (Provocholine, Roche Laboratories, Nutley, N.J.) was administered with a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, Pa.) connected to a Rosenthal-French dosimeter (Laboratory for Applied Immunology, Baltimore, Md.) powered by an oxygen tank with a pressure of 20 psi and calibrated to deliver 15 μ l per breath. The result of this test was expressed in terms of the provocation dose necessary to decrease forced expiratory volume in 1 second (FEV₁) by 20% (PD₂₀), given in cumulative breath units (1 unit = one inhalation of aerosol from a 1 mg/ml solution of methacholine).

Specific bronchial provocation tests (BPTs) were done in subject 1 when he was absent from work, free of symptoms, and not receiving any medication that could affect the test results. The aerosolized particles were generated by a continuous pressurized DeVilbiss 646 nebulizer with a fitted output of 0.28 ml/min, and inhaled by tidal breathing for 2 minutes. A control challenge with PBS, performed before antigen challenge, caused no reaction. Skin prick tests with 10-fold dilutions (from 10⁻⁶ to 10⁻¹) of carmine and cochineal extracts were performed to enable selection of a safe initial dose for the BPT (concentration of allergen that produces a 3 mm wheal); this concentration was 0.1 mg/ml for carmine extract and 1:100 wt/vol for cochineal extract. The following inhalation tests were conducted on different days: carmine in stepwise concentrations of 0.1, 1, and 10 mg/ml; cochineal, 1:100 and 1:10 wt/vol; carminic acid, 10 mg/ml; and annatto, 1:10 wt/vol. Baseline pulmonary function measures of FEV₁ and forced vital capacity were performed and repeated at 5, 10, 15, 20, 30, and 60 minutes and then hourly until 10 hours after each challenge. A positive response was defined as a fall of 20% or more in FEV₁. Three nonexposed subjects with asthma who had PD₂₀ values of 20, 25, and 50 cumulative breath units, respectively, were also challenged with the maximum concentration of carmine (10 mg/ml) and cochineal (1:10 wt/vol) extracts after they had given informed consent.

Oral challenge test

To determine whether there was some type of allergic response after ingestion of carmine, a double-blind, placebo-controlled, oral challenge test was performed on the worker with asthma (subject 1). The cochineal red synthetic dye (CI 16255), very similar in color but chemically unrelated to carmine, was used as placebo at a maximum concentration of 1 mg/ml. Ten milliliters of either placebo or freshly prepared solutions of carmine at increasing concentrations of 0.1, 1, and 10 mg/ml in water were administered orally. The admissible daily ingestion for carmine, up to 5.0 mg per kilogram of body weight, was not exceeded. Before and after each oral challenge, pulmonary function measures of FEV₁ and forced vital capacity were performed at 5, 10, 15, 20, 30, and 60 minutes. If no response was observed at that time, the following dose was given. During the period of

observation, which lasted 2 hours after the last dose was administered, the appearance of respiratory, skin, or gastrointestinal symptoms was assessed.

RESULTS

Clinical questionnaire

Of the nine current workers, a process worker had work-related symptoms of rhinitis and asthma. This worker had sneezing, rhinorrhea, nasal and ocular itching, dyspnea, wheezing, unproductive cough, and chest tightness on exposure to carmine. These symptoms became worse at home, especially during the night and early morning. He markedly improved on weekends and vacations. Another employee, a cleaner, had symptoms of rhinitis within a few minutes after exposure to carmine dust. The former employee, a process worker, had left work 2 years ago after work-related respiratory symptoms developed and were diagnosed in our department as carmine-induced asthma.¹² He had complete resolution of his symptoms shortly after leaving his workplace, and he has remained free of symptoms until now. ■Table I illustrates the clinical data of the study subjects.

TABLE I. Clinical characteristics of study subjects

Subject No.	Age (yr)	Sex	Occupation (yr employed)	Symptoms at work (duration in mo)	Patient/Family atopy	Smoking (pack yr)	Total IgE (kU/L)
1	27	M	Plant worker (2)	RC/A (20)	No/No	9	433
2	40	F	Cleaner (3)	R (24)	No/No	0	61.9
3	24	F	Laboratory technician (3.5)		No/No	6	64.5
4	26	F	Office worker (3.5)		No/No	8	46.8
5	36	M	Chemist (5)		No/Yes	13.5	304
6	24	F	Office worker (2)		No/No	9	14.4
7	17	M	Plant worker (0.25)		No/No	1.5	22.1
8	29	F	Laboratory technician (4.5)		No/No	16.5	9.35
9	17	M	Plant worker (1.5)		No/No	1.5	2.54
10	23	M	Ex-plant worker (1)	R/A* (7)	No/No	9	90.6

A, Asthma; R, rhinitis; RC, rhinoconjunctivitis.

*After removal from exposure, he was free of symptoms.

PEFR recordings

Baseline PEFR recordings were within normal limits (>80% predicted value) in all workers.

Serial PEFR measurements in subject 1, with work-related symptoms of asthma, showed significant drops (>25%) from baseline values and variability higher than 20% on the days he was working with carmine.

Skin tests

The results of skin prick tests with cochineal and carmine extracts were positive only in subjects 1, 2, and 10. Skin prick test results with cochineal-derived products are shown in Table II. Results of skin prick tests with curcuma and annatto extracts were negative in all the employees, as were results of tests with common inhalant allergens. Control patients showed no reaction to any of these dyes.

TABLE II. Results of skin prick tests to cochineal-derived products

Subject No.	<u>Perpendicular diameters of the wheal (mm)</u>				
	Carmine (10 mg/ml)	Cochineal (1:10 wt/vol)	CA (10 mg/ml)	CA-HSA conjugate	Histamine (10 mg/ml)
1	15 × 7 ps	10 × 8 ps	0	0	5 × 5
2	4 × 4	3 × 3	0	0	7 × 6
3	0	0	0	0	6 × 6
4	0	0	0	0	7 × 7
5	0	0	0	0	7 × 6
6	0	2 × 2	0	0	6 × 6
7	0	0	0	0	7 × 7
8	0	0	0	0	6 × 6
9	0	0	0	0	7 × 7
10	8 × 6	6 × 6	0	0	8 × 7

CA, Carminic acid; ps, pseudopods.

Specific IgE determination

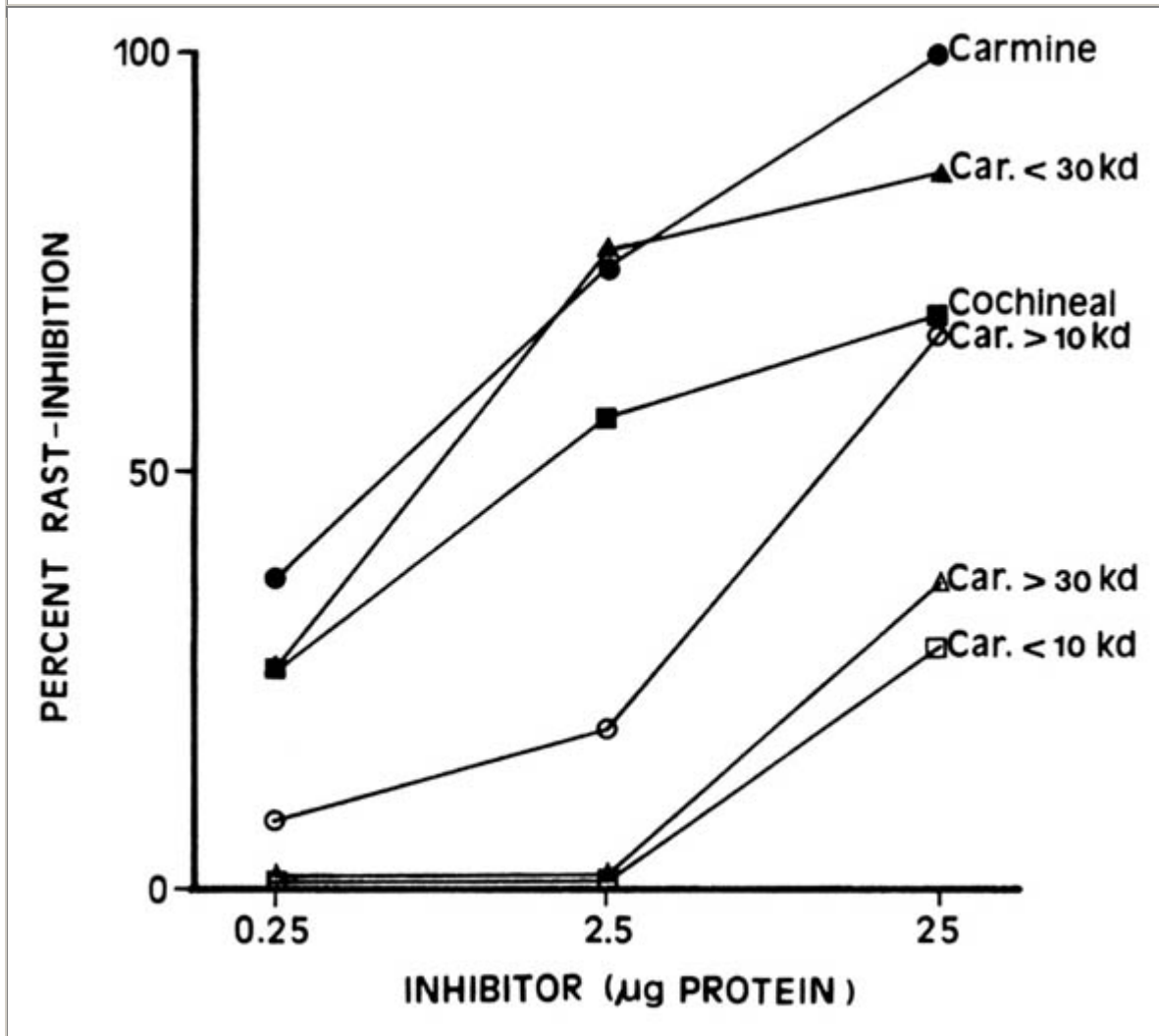
Specific IgE antibodies against carmine and cochineal were found in only subject 1, the only one who currently had work-related asthma. Specific IgE binding to carmine in this patient was 27.35% of the total activity added, and IgE binding to cochineal was 18% of the total activity added. However, RAST to the carminic acid-HSA conjugate was negative (<1%). All of the other employees, including subject 10, who had had occupational asthma 2 years previously, and the control subjects, did not show binding greater than 2%.

RAST inhibition

With the serum of subject 1 it was found that IgE binding to carmine disks could be significantly inhibited by the following allergens, in decreasing order of potency: complete carmine extract, carmine fraction of MW less than 30 kd, cochineal extract, and carmine fraction of MW higher than 10 kd. On the contrary, with carmine fractions of MW higher

than 30 kd and less than 10 kd, only a small inhibition was observed with the highest concentrations of both extracts. The results of inhibition studies are presented in ■Fig. 2.

FIG. 2. RAST inhibition with carmine disks and serum from subject 1. Amount of inhibitor (μg protein) added per 50 μl of serum is illustrated on the x axis.



Specific IgG subclasses

IgG antibodies specific to carmine and cochineal, mainly the subclasses IgG₁, IgG₃, and IgG₄, were found in all the employees surveyed. These values are shown in ■Tables III and ■IV, respectively. There were no significant differences between subjects with symptoms and those without symptoms for any of these subclasses. A statistically significant correlation (Pearson's correlation coefficient [r]) between the values (after logarithm transformation) of each specific IgG subclass to carmine and the corresponding values to cochineal was found: $r = 0.83$ and $p < 0.01$ for IgG₁ (■Fig. 3); $r = 0.89$ and $p < 0.01$ for IgG₂ (deviated data from subject 8 were eliminated); $r = 0.84$ and $p < 0.01$ for IgG₃; and $r = 0.69$ and $p < 0.05$ for IgG₄. The specificity of the ELISA for carmine and cochineal was demonstrated by the ELISA inhibition assays, with a maximum inhibition up to 90% with the corresponding allergen.

TABLE III. Specific IgG subclass levels to carmine by ELISA (optical density at 492 nm)

Subject No.	IgG ₁	IgG ₂	IgG ₃	IgG ₄
1	2.910	0.422	1.929	3.200
2	3.200	0.350	3.400	0.404
3	0.738	0.293	0.975	2.100
4	3.600	0.555	>4.000	0.422
5	3.300	0.717	>4.000	2.010
6	1.179	0.598	2.100	0.608
7	2.200	0.405	>4.000	0.864
8	2.900	3.400	>4.000	>4.000
9	3.200	0.676	3.300	0.344
10	1.562	0.563	1.143	1.422
Controls*	0.53 ± 0.13	0.22 ± 0.03	0.78 ± 0.14	0.44 ± 0.06

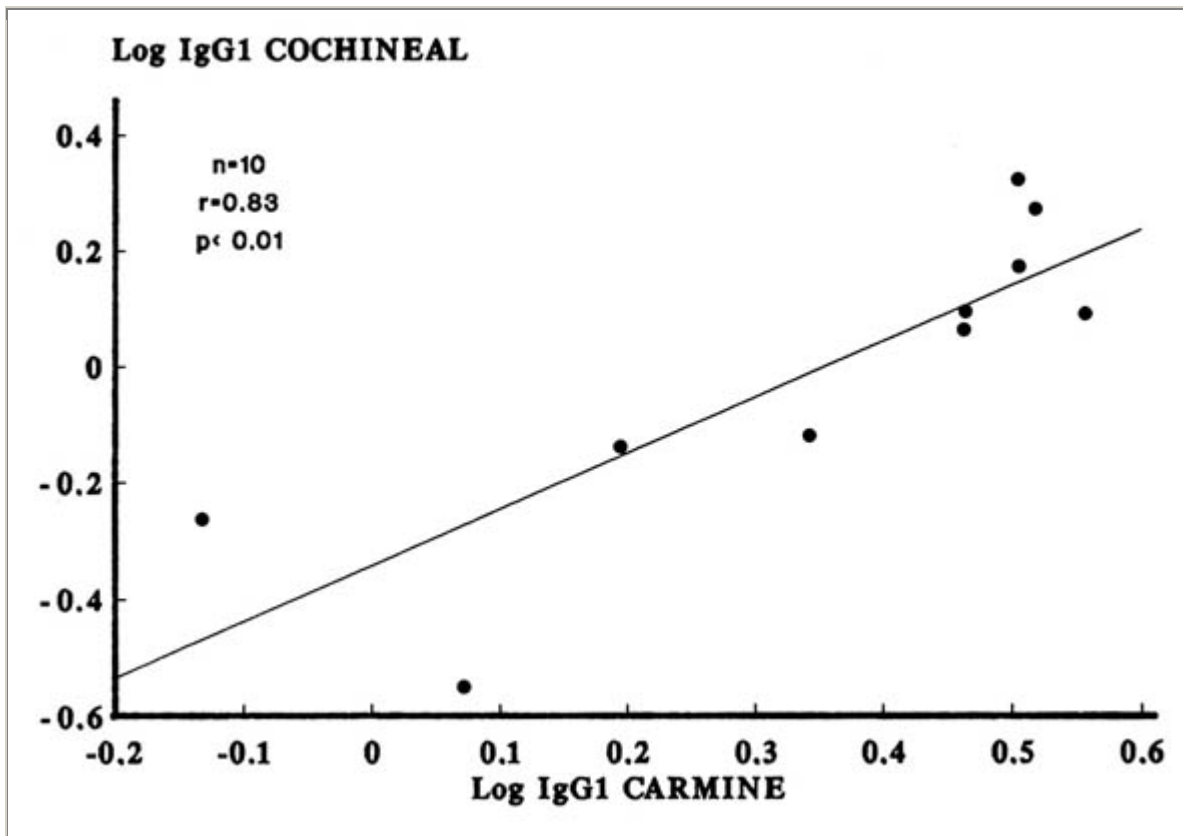
*Mean values ± SD from seven nonexposed persons.

TABLE IV. Specific IgG subclass levels to cochineal by ELISA (optical density at 492 nm)

Subject No.	IgG ₁	IgG ₂	IgG ₃	IgG ₄
1	1.255	0.313	1.002	0.525
2	1.489	0.386	1.882	0.388
3	0.547	0.254	1.054	1.415
4	1.222	0.582	3.000	0.237
5	1.857	0.818	1.920	0.709
6	0.888	0.446	1.112	0.295
7	0.752	0.310	2.000	0.874
8	1.158	0.549	1.406	0.871
9	2.100	0.549	1.958	0.355
10	0.719	0.539	0.677	0.429
Controls*	0.55 ± 0.11	0.27 ± 0.03	0.61 ± 0.08	0.31 ± 0.03

*Mean values ± SD from seven nonexposed persons.

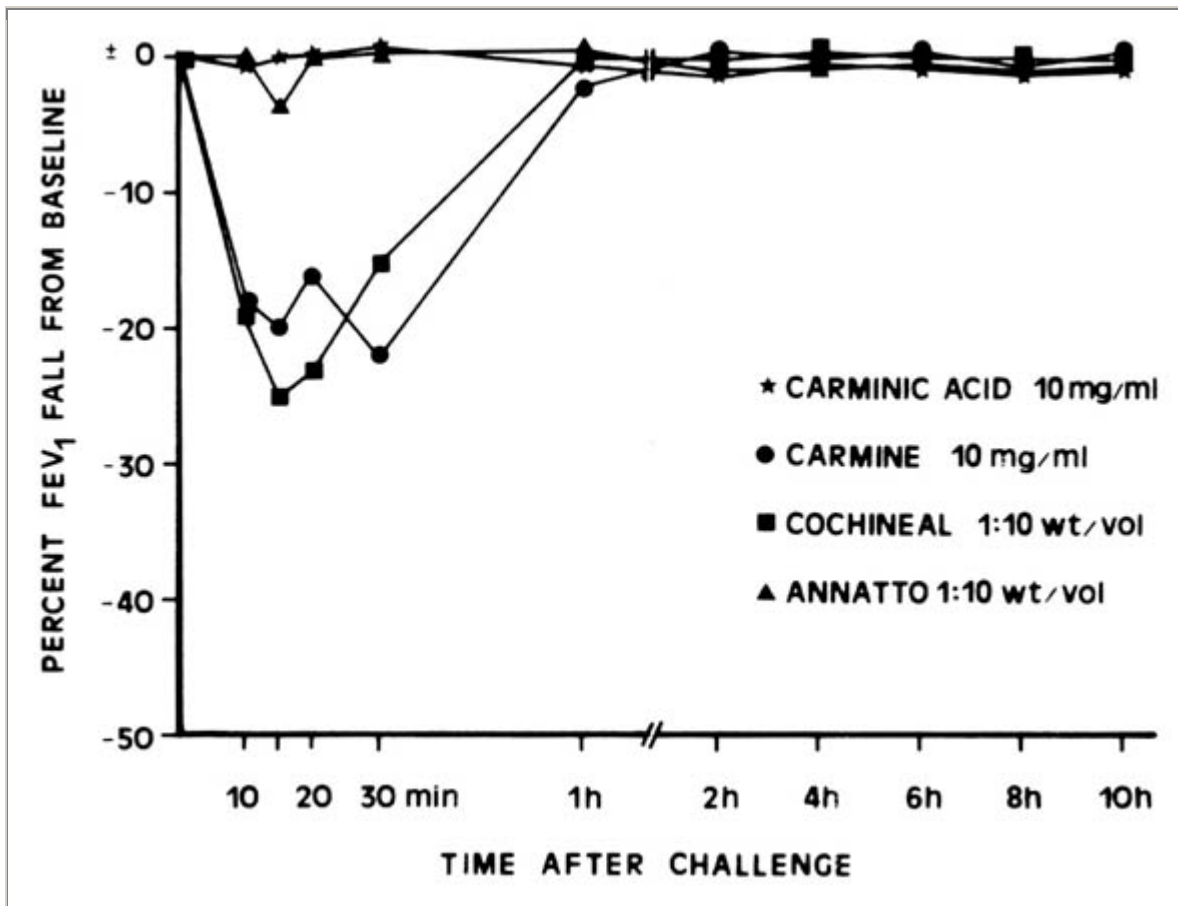
FIG. 3. Correlation between IgG₁ specific to carmine and IgG₁ specific to cochineal.



Bronchial provocation tests

Methacholine inhalation test revealed severe bronchial hyperresponsiveness in subject 1, with a PD_{20} value of 20 cumulative breath units. Specific BPTs with both carmine (10 mg/ml) and cochineal (1:10 wt/vol) elicited isolated immediate responses. On the contrary, no response was observed after BPT with carminic acid (10 mg/ml) and annatto dye (1:10 wt/vol). The results of BPTs are shown in Fig. 4 Control subjects showed no response to the BPT. with carmine or cochineal.

FIG. 4. Results of specific bronchoprovocation tests in subject 1.



Oral challenge test

After the maximum dose of 100 mg of carmine in aqueous solution was administered to subject 1, no symptoms suggestive of an allergic or adverse reaction were observed.

DISCUSSION

In this study we investigated the 10 workers at a natural dye-processing factory; one of them had previously left this plant after carmine-induced asthma developed. A prior evaluation of this former worker when he was experiencing symptoms had already confirmed occupational asthma by specific BPT with carmine, ¹² a dual response was shown, and both positive skin prick and histamine release responses to carmine were obtained. At the time of the present study we could not demonstrate specific IgE antibodies against carmine in this subject; despite this, results of skin prick testing were still positive. Nevertheless, specific IgE antibodies might have fallen after cessation of exposure, as has been described for other occupational sensitizers. ¹³ Among the nine current employees, a plant worker had rhinitis and asthma when processing carmine and a cleaner had work-related rhinitis, which was possibly caused by carmine inhalation, although this could not be demonstrated, because the cleaner refused provocation studies. Carmine-induced asthma was confirmed in the current worker with asthma by BPT; nonirritating concentrations of both carmine and cochineal caused an immediate response. A positive methacholine inhalation test result and the significant changes in PEFR recordings when he was processing carmine supported the diagnosis of occupational

asthma. Moreover, positive skin prick test and RAST results to carmine and cochineal in this subject strongly suggest an IgE-mediated pathogenesis. It is worth noting that the three employees described with work-related respiratory symptoms had a high degree of carmine exposure because of their occupations. However, all employees at this factory, regardless of their occupations or whether they had symptoms, had high levels of specific IgG subclass antibodies to carmine and cochineal, probably as a consequence of the highly carmine-contaminated environment to which they were exposed.

Burge et al. ⁶ in 1979 first reported two patients with work-related asthma caused by carmine inhalation, in whom dual responses to the BPTs with carmine and cochineal extracts were observed. As opposed to our patient, oral challenge with cochineal extract or Campari (a carmine-containing beverage) provoked gastrointestinal symptoms in one of these earlier patients and asthma in both. However, specific IgE antibodies were not found in either patient by skin testing or RAST. Tenabene et al. ⁷ described three workers with carmine-induced asthma confirmed by cabin exposure test; two of them had positive reactions to skin tests, although specific IgE was not demonstrated by RAST. Durham et al. ¹⁴ described a worker with a dual response after BPT with carmine, who showed an increase in histamine airway responsiveness after specific BPT, but no immunologic data of this patient were shown. Christiansen et al. ⁸ and more recently Dietemann-Molard et al. ⁹ described several workers in food and food additive factories with extrinsic allergic alveolitis caused by carmine inhalation, with demonstration of precipitins to carmine in some of these cases. It is surprising that although cochineal extract and carmine have been extensively used, few studies have reported respiratory symptoms from these compounds and in no study was specific IgE demonstrated until now. This low reported rate may be due to the fact that the development of suitable immunologic techniques have coincided with a decline in the use of carmine because of its higher price in relation with synthetic dyes. On the other hand, there was no evidence of sensitization to curcuma or annatto dyes in any of the workers. We found only one report of a patient in whom anaphylaxis caused by annatto dye developed after ingestion of a cereal formula, and an allergen of 50 kd was demonstrated by immunoblotting. ¹⁵

It is known that the Spanish discovery of America heralded a new era in the exploitation of insect pigments and provided Europe with the first imports of American cochineal as used today; this promptly became the most widely traded product of the Spanish Indies. ¹⁶ The coloring principle of carmine is chiefly carminic acid. Commercial carmines also contain proteinaceous material derived from the source insect during the extraction of the pigment, and the protein content in carmine for food and beverage uses is 15% to 25%. ¹⁶ These coccoid-derived proteins are potential aeroallergens, similar to other arthropod-derived proteinaceous material. ¹⁷ Until now few attempts have been made to characterize the protein components derived from the insect or to assess the way in which carminic acid may be associated with them. With regard to this it has been shown that although purified carminic acid is freely dialyzable, a substantial proportion of the carminic acid content of carmine does not diffuse across conventional membranes, suggesting some form of combination between the hydroxyanthraquinone (carminic acid) and high-MW components. ¹⁶ Thus carmine is an interesting etiologic agent of occupational asthma because it has both low- and high-MW components that at least theoretically may act as sensitizers. Because of its low MW, carminic acid could act as a hapten and combine with a protein to elicit an immunologic response, and it might involve new antigenic determinants of the protein. Thus the specificity of IgE antibodies against carmine or cochineal could be protein specific, hapten specific (the protein acting as a carrier), or modified protein (new determinants) specific. In the patient we studied both the negativity

of skin test and RAST results with carminic acid-HSA conjugate and the absence of response to the BPT with carminic acid are against the hypothesis of hapten-specific antibodies. On the contrary, our studies pointed out that he was sensitized to a high-MW antigen (between 10 and 30 kd) present in both *D. coccus* (cochineal) and carmine. The strong dose-dependent inhibition of carmine RAST by cochineal and the good correlation between specific IgG subclass values to carmine and cochineal support the hypothesis that the main antigen or antigens are shared by carmine and cochineal.

In conclusion, our results demonstrate that carmine may induce antibody responses, most likely IgE mediated in workers with symptoms of occupational asthma. However, the differences in the allergy test results observed in some of the patients with carmine-induced asthma described in the literature might be due to the fact that several components in carmine could be acting as the etiologic agents.

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